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BIOLOGICAL DENITRIFICATION OF HIGH EXPLOSIVES PROCESSING WASTEWATERS

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ABSTRACT

Previous work in our laboratory over the past four years has shown that the high explosives hexahydro-1,3,5,-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) can be readily destroyed by alkaline hydrolysis. The hydrolysis process produces large quantities of concentrated but conventional wastewaters, containing acetate, formaldehyde, formate, ammonia and nitrite. To treat these wastewaters, a denitrifying (anoxic) biological process was developed that converts the hydrolysates to harmless endproducts, such as N_2 and CO_2. The nitrite is produced during the hydrolysis process as the electron acceptor, but additional nitrite is required to completely oxidize the carbon compounds. Over 90 percent of each organic carbon source can be removed in a packed-bed, upflow reactor in 3 hours hydraulic retention time. Formaldehyde and acetate are first degraded at approximately the same rate, and formate is degraded more slowly. Results closely match the stoichiometry predicted by the empirical redox equations describing the process. © 1997 IAWQ. Published by Elsevier Science Ltd

KEYWORDS

Hydrolysates (hydrolysis byproducts); biological denitrification; hexahydro-1;3;5;-trinitro-1;3;5-triazine(RDX); octahydro-1;3;5;7-tetranitro-1;3;5;7-tetrazocine(HMX).

INTRODUCTION

Explosives have been manufactured in the United States and other countries for many decades. Among all the high explosives (HE) that are manufactured, RDX (Hexahydro-1,3,5,-trinitro-1,3,5,-triazine), HMX (Octahydro-1,3,5,7-Tetranitro-1,3,5,7-tetrazocine) and TNT (2,4,6,-trinitrotoluene) are the most common explosives and the most important high explosives used by the U.S. and European munitions industries. Mixtures of RDX and HMX or TNT are major components in both conventional and nuclear weapons. RDX is classified as a possible human carcinogen (Class C) by the US EPA, and has various effects on mammals, fish, protozoa (McLellan *et al.*, 1988).

Recent increases in waste production due to the end of the cold war have increased disposal and treatment problems. The United States and other countries are destroying large quantities of weapons (demilitarization), which creates a need for safe and reliable disposal technologies for energetic materials. In the past, wastewater management disposed the RDX wastes in lagoons. Recent work in our laboratory (Heilmann *et al.*, 1996) combined the techniques of activated carbon adsorption and alkaline hydrolysis to produce a new process that has the advantages of carbon adsorption without the regeneration or disposal

problems associated with HE-laden carbon. Figure 1 shows the process. The RDX contaminated wastewater is first adsorbed onto granular activated carbon to concentrate RDX waste on the carbon which reduces the volume of material to be treated. The laden carbon is treated with alkaline hydrolysis which effectively regenerates it. Alkaline hydrolysis of RDX produces 1.6 M NO_2^-, 1.5 M $HCOO^-$, 0.1 M CH_3COO^-, 1.1M HCHO, 0.9 M NH_3, 1.1 M N_2O, and 0.34 M N_2 per mole of RDX hydrolyzed. HMX hydrolyzes at slower rates than RDX, but produces similar byproducts with slightly different stoichiometry.

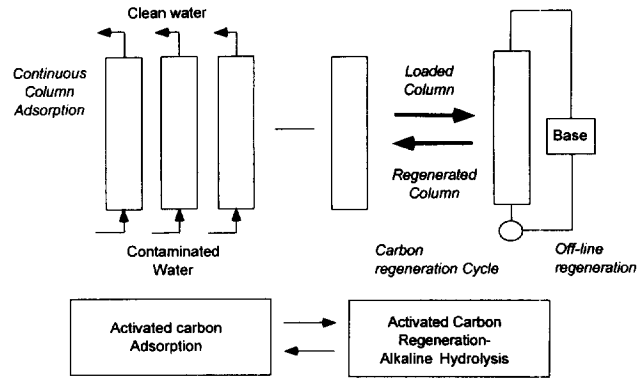

Figure 1. The process of treating high explosives using activated carbon adsorption and alkaline hydrolysis (Heilmann *et al.*, 1996).

The hydrolysates require further treatment before discharge to the environment. The easily degradable, low molecular weight, organic byproducts and the presence of a large amount of nitrite suggested a denitrifying anoxic biological process. Such a process could oxidize the organic compounds to CO_2 and reduce the nitrite to nitrogen gas to eliminate a potential nutrient problem. This paper describes our research to develop and understand this process. Synthetic hydrolysates were used that closely simulate actual hydrolysates. The process was developed in a step-wise fashion, by gradually increasing the number of organic compounds and the mass of electron acceptor.

MATERIALS AND METHODS

Continuous flow experiment and denitrifying culture

A two hundred mm Plexiglas column with an internal diameter of 25 mm was packed with 42 grams of size 13 (diameter 2 mm) silicon tubing, cut to lengths of 1-2 mm. and used for treating the hydrolysate. The column has an internal diameter of 25 mm resulting in an empty bed volume of 98.2 ml. The inlet and outlet were equipped with stainless steel screens to retain the packing material. The column was operated at room temperature (26-27 °C) in an upflow mode and fed by a cartridge pump. Feed solution was mixed in a 2 L flask every other day. Tygon tubing was used to deliver the feed from a preparation flask to the reactor and to minimize sorptive interactions. The flask tubing was flushed with a dilute acid solution every other day to prevent growth of bacteria outside the reactor.

Anaerobic digester sludge was collected from the Hyperion wastewater treatment plant in El Segundo, CA. The mixed cultures were diluted with oxygen free phosphate buffer, filtered, and incubated for three weeks in a minimal medium with ethanol, potassium nitrate, phosphate buffer. The column was inoculated by injecting 1 ml of mixed culture into the lower quarter of the reactor that was prefilled with feed solution. The feed flow was initiated two weeks after inoculation to allow for cultural growth and attachment to packing material. The liquid growth medium contained K_2HPO_4 5 g/L, $NaH_2PO_4 \cdot H_2O$ 2.88 g/L, NH_4Cl 0.2 g/L, $MgCl_2 \cdot H_2O$ 0.1 mg/L, $CaCl_2 \cdot 2H_2O$ 0.04 g/L, $Na_2SO_3 \cdot$ 0.02 g/L. This basal medium was supplemented with hydrolysates and a trace nutrient solution ($FeCl_3$ 3.9 mg/L, $MnCl_2$ 0.95 mg/L, $ZnCl_2$

0.66 mg/L, $CoCl_2 \cdot 6H_2O$ 0.58 mg/L, $CuCl_2 \cdot H_2O$ 0.30 mg/L, $Na_2Mo_4 \cdot 2H_2O$ 0.46 mg/L, $Na_2B_4O_7 \cdot 10H_2O$ 0.24 mg/L). The flowrate of feeding solution was 0.3 ml/L. except the kinetic experiment (0.6 ml/min). Phosphate buffer was provided to maintain pH between 7-8. The influent was sampled directly from the feed solution and the effluent was sampled by collecting the solution as it exited from the reactor.

Analysis of hydrolysates and high explosives

NO_2^- (nitrite) and $HCOO^-$ (formate) were analyzed using a Dionex Ion Chromatograph (basic chromatography module CMB-2, gradient pump GPM-1; Dionex, Sunnyvale, CA) with suppressed conductivity detection (conductivity detector CDM-1). An Ion Pac AS9-SC analytical column (4 mm I.D.) was used with a subsequent suppressor column.

For detection of formaldehyde, we modified the detection technique of Kuwata *et al.* (1979). Formaldehyde in a sample was allowed to react with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone, and solid phase extraction method (Richard and Junc, 1986) was used to change solvent from water to acetonitrile. This solution was analyzed with high performance liquid chromatography (HPLC) with an Adsorbosphere C-18 micron reversed-phase column (Altech, Deerfield, IL)

Acetate ion ($CH3COO^-$) analysis was performed on a Hewlett Packard Gas Chromatograph (model 5890; Avondale, PA). A glass column with 15% SP-1200/1% H_3PO_4 on 100/200 mesh chromosorb (Supelco, Bellefonte, PA) and a guard column were used to separate peaks. Oven temperature and injection temperature were 120°C, and detector temperature was 200°C.

MICROBIOLOGY AND STOICHIOMETRY

Denitrification has become a relatively well known process (Gayle *et al.*, 1989; Knowles, 1982) to remove nitrogenous compounds biologically from wastewater. Nitrate (NO_3^-) is usually used as the terminal electron acceptor and it is sequentially reduced to NO_2^- and finally to N_2 (Payne, 1973). An easily degradable carbon source, such as methanol, ethanol or acetic acid are frequently added when no carbon source is available, because they are relatively inexpensive and have low solids yields. If methanol is used as a carbon source and nitrate is an electron acceptor, the stoichiometric relationships describing bacterial energy equations is written as follows:

$$6\ NO_3^- + 5\ CH_3OH \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + OH^- \quad (1)$$

In the treatment of wastewaters that are deficient in organic carbon, methanol has been used as a carbon source, but for denitrification of hydrolysates, the formate, formaldehyde, and acetate are used as the carbon source. The nitrite, produced from RDX hydrolysis, is used for final electron acceptor instead of nitrate. Nitrite (an electron acceptor) is reduced to gaseous nitrogen in accordance with the following half equation:

$$NO_2^- + 4\ H^+ + 3\ e^- \rightarrow 0.5\ N_2 + 2\ H_2O \quad (2)$$

The half equations for the carbon sources are shown in Equations 3 to 5.

$$HCOO^- \rightarrow CO_2 + H^+ + 2\ e^- \quad (3)$$

$$HCHO + H_2O \rightarrow CO_2 + 4\ e^- + 4\ H^+ \quad (4)$$

$$CH_3COO^- + 2\ H_2O \rightarrow 2\ CO_2 + 8\ e^- + 7\ H^+ \quad (5)$$

Equations 6 to 8 show the stoichiometric relationships describing bacterial energy production between nitrite and each carbon source.

$$NO_2^- + 1.5\ HCOO^- + 2.5\ H^+ \rightarrow 0.5\ N_2 + 1.5\ CO_2 + 2\ H_2O \quad (6)$$

$$NO_2^- + 0.375\ CH_3COO^- + 1.375\ H^+ \rightarrow 0.5\ N_2 + 0.75\ CO_2 + 1.25\ H_2O \quad (7)$$

$$NO_2^- + 0.75\ HCHO + H^+ \rightarrow 0.5\ N_2 + 0.75\ CO_2 + 1.25\ H_2O \quad (8)$$

RESULTS AND DISCUSSION

Denitrification of nitrite using acetate and formate

As we mentioned earlier, Heilmann *et al.* (1996) found that combined techniques of activated carbon adsorption and the alkaline hydrolysis of RDX yields 1.6 M NO_2^-, 1.5 M $HCOO^-$, 0.1 M CH_3COO^-, 1.1M HCHO, 0.9 M NH_3, 1.1 M N_2O, and 0.34 M N_2 per mole of RDX hydrolyzed. Equation 9 shows this relationship:

$$1\ RDX\ (C_3H_6N_6O_6) \rightarrow 1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 0.9\ NH_3 + 1.1\ N_2O + 0.34\ N_2 \uparrow \quad (9)$$

For simplicity, we began the post-treatment of hydrolysates of RDX using only acetate and formate. Equation 10 shows the empirical stoichiometric relationship for this system.

$$1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 2.9\ H^+ \rightarrow 0.33\ NO_2^- + 0.63\ N_2 + 1.7\ CO_2 + 2.33\ H_2O \quad (10)$$

From the stoichiometric relationship, formate and acetate should be oxidized to CO_2, and the nitrite concentration should be decreased by approximately 20%. The reactor system was operated for three months to demonstrate this condition. Figure 2 shows the percentage removals of nitrite and formate of this system. At this time of these experiments, we were not monitoring acetate removal. As shown in Figure 2, formate removal was greater than 90%, but nitrite removal was about 50 %. This lower removal of nitrite than theoretical value (79 %) can be explained by the cell synthesis.

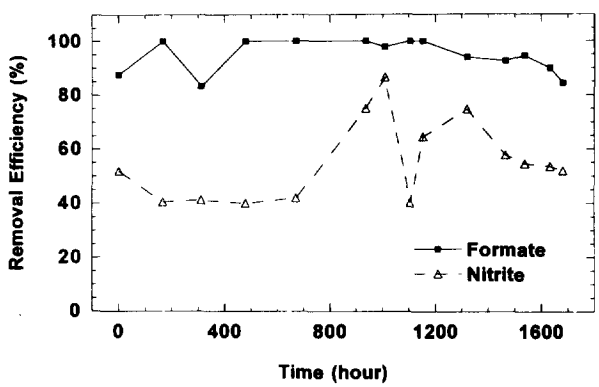

Figure 2. Removal efficiency of formate and nitrite (acetate was not monitored in this period).

Formaldehyde addition

After developing stable conditions with acetate and formate, formaldehyde was added, which simulates actual hydrolysates. The first step was to determine the stoichiometric relationships including formaldehyde,

however, insufficient nitrite exists to complete the oxidation of all three carbon sources. Therefore, additional nitrite must be added. To determine the required nitrite, the stoichiometric equations are written as follows:

$$(1.13\ NO_2^-) + 1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 4.334\ H^+ \rightarrow 1.366\ N_2 + 2.8\ CO_2 + 4.16\ H_2O \quad (11)$$

Less than the stoichiometric amount will be required due to cell synthesis. Therefore, it was necessary to experimentally determine the required nitrite. Equation 12 shows the stoichiometry for best overall removal.

$$(1.0\ NO_2^-) + 1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 4.37\ H^+ \rightarrow 1.36\ N_2 + 2.8\ CO_2 + 4.16\ H_2O \quad (12)$$

Figure 3 shows percent removal of each hydrolysate in the bioreactor with this ratio. During one month, we obtained over 90 % removal of all hydrolysates. This result was confirmed with total organic carbon (TOC). As in shown in Figure 4, influent TOC was about 100 mg/L, and effluent TOC was about 10 mg/L. Table 1 also shows the match between "Calculated TOC" and "Measured TOC". In this table, "Calculated TOC" indicated all the sum of C-carbon sources. All these data show how the denitrification performed well according to empirical stoichiometry.

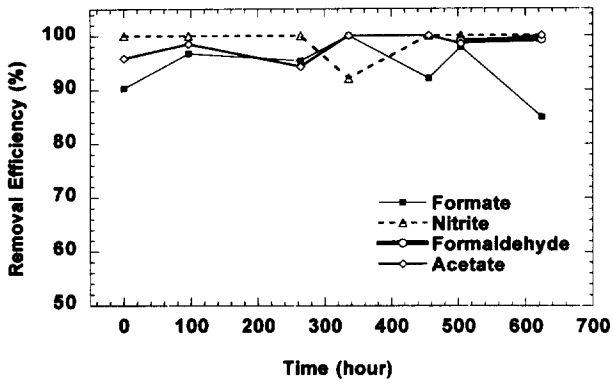

Figure 3. Removal efficiency of each hydrolysate after adding formaldehyde (formaldehyde was analyzed later in this experiment).

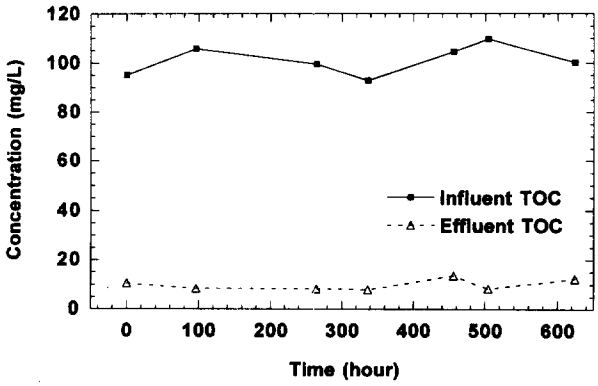

Figure 4. Comparison of influent and effluent total organic carbon.

Table 1. Comparison between "calculated TOC" and "measued TOC"

(mg/L)	C-Formate	C-Formaldehyde	C-Acetate	*Calculated TOC	Measured TOC
Influent	49.9	35.6	6.5	92.1	100.2
Effluent	7.5	0.3	0.0	7.8	12.3

* In calculation of TOC, C-methanol (8.41 mg/L) in formaldehyde stock solution was not considered.

Degradation kinetics of carbon sources

In order to evaluate the reactor's preference among the three different carbon substrates, degradation efficiency was measured along the length of the reactor for each carbon source. The data were collected with the reactor operating at 0.6 ml/min and the influent concentration ratio of 166.2 mg of NO_2^--N, 97.9 mg $HCOO^-$-C, 71.8 mg of HCHO-C, and 13.1 mg of CH_3COO^--C in 1 L. Multiple samples were taken and averaged over time from the inlet (x = 0 cm), the outlet (x = 20 cm) and 4 to 5 ports along the length. The degradation for the carbon sources is presented in Figure 5. The results indicated that acetate and formaldehyde are degraded first, and formate is degraded last. This result seems reasonable because formaldehyde is a less oxidized form than formate, so it is more easily oxidized than formate. Acetate and formaldehyde degradation rates are almost equal.

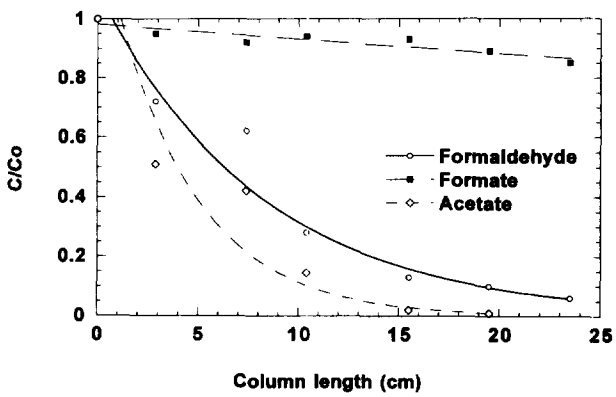

Figure 5. Degradation profile of carbon sources along the column length (nitrite removal is 78%, flowrate is 0.6 mL/min and reactor inlet is located at 0 cm),

pH control and maximum treating capability

The hydrolysates of RDX contain high pH and salinity because of alkaline hydrolysis, and must be neutralized before entering the bioreactor system. In denitrifying systems, the denitrification rate is positively related to pH, with an optimum in the range of 7.0 to 8.0 (Knowles, 1982). In order to keep this range of pH, phosphate buffer was used in this case.

Another concern is that alkalinity is produced during conversion of nitrite to nitrogen gas, resulting in a pH increase. Table 2 shows that the pH increased with increasing influent hydrolysate concentrations. The results show the phosphate buffer concentration was not enough for high influent concentrations of hydrolysates. For a full scale or pilot scale system, acid is needed for pH control. We calculated the acid amount needed using Equation 12. From the stoichiometric relationship, 0.037 M of acid(HCl) is needed to maintain neutral pH if we treat hydrolysates containing N(nitrite): C(acetate) : C(formaldehyde) : C(formate) in the ratio of 1 g: 0.08 g: 0.43 g: 0.59 g per 1L.

Table 2. pH changes of influent and effluent samples as the concentrations of feeding solution change*

C-Acetate (mg/L)	: C-Formaldehyde (mg/L)	: C-Formate (mg/L)	: N-Nitrite (mg/L)	Influent pH	Effluent pH
6.53	35.9	49.0	83.1	6.95	7.15
13.1	71.8	97.9	166.2	6.95	7.48
26.1	143.6	195.8	332.3	6.94	7.94

* The stoichiometric relationship is $(1.0\ NO_2^-) + 1.6\ NO_2^- + 1.5\ HCOO^- + 0.1\ CH_3COO^- + 1.1\ HCHO + 4.37\ H^+ \rightarrow 1.36\ N_2 + 2.8\ CO_2 + 4.16\ H_2O$

Finally, we investigated maximum treating capability of the biological reactor as we increased influent hydrolysate concentrations. As shown in Figure 6, the concentration of each hydrolysate increased up to 4 times original concentration in which the compositions are 83.1 mg/L of NO_2^--N, 49.0 mg/L of $HCOO^-$-C, 35.9 mg/L of HCHO-C, and 6.53 mg/L of CH_3COO^--C. However, the removal efficiency of each compound was not recovered even after two months. The result suggests that this reactor can treat hydrolysates up to 166.2 mg/L of NO_2^--N, 97.9 mg/L $HCOO^-$-C, 71.8 mg/L of HCHO-C, and 13.1 mg/L of CH_3COO^--C per day. Over this range of concentrations, the effluent pH increased above pH 8, which is beyond the optimum pH range (7.0-8.0) for denitrification and reduce treatment efficiency.

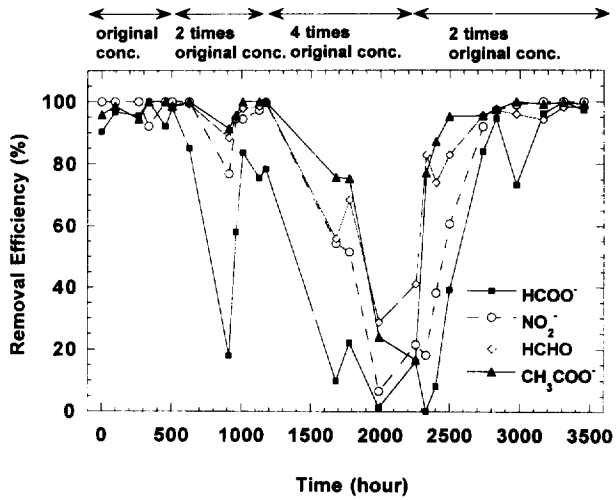

Figure 6. The change of removal efficiency vs. change of hydrolysate concentration.

CONCLUSION

The application of biological denitrification for treating synthetic hydrolysates of RDX was demonstrated. The results show that post-denitrification of RDX hydrolysates is a feasible process. Results obtained during two years study shows that:

- Nitrite, one of RDX hydrolysate byproducts can be used as the electron acceptor. Three organic RDX/HMX hydrolysate byproducts (acetate, formaldehyde, formate) were effectively oxidized. The removal efficiency of each byproduct was over 90%, and results were verified with TOC.
- Experimental results closely matched calculated stoichiometric conversions.
- The volumetric removal rate was as high as 166.2 mg/L NO_2^--N per day with existing carbon sources.
- Acetate and formaldehyde were preferentially degraded over formate.
- In a full scale or pilot plant study, additional acid should be added for neutralization before and after denitrification in order to prevent discharge of high pH.

The study shows the application of biological denitrification for treating byproducts as the post-denitrification of the hydrolysates of RDX is a feasible process in spite of the complexity of the system. These results may be applicable to other industrial wastewaters containing nitrite or nitrate, and multiple carbon sources. A pilot scale research is underway at the Pantex Plant in Amarillo, TX. Research is also underway to treat hydrolysates from trinitrotoluene (TNT).

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